*Penumala Sony et al, 2026, Vol.14, No.4: 67-72*

**International Journal of Chemistry and Pharmaceutical Sciences**

https://doi.org/10.30904/j.ijcps.2026.5010 | E-ISSN: 2321-3132

# Pharmacological Screening Activity of Selected Plant Extract in Mice Model

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Received: 29-01-2026 | Revised: 19-02-2026 | Accepted: 27-03 2026 | Published: 30-04-2026

**Abstract:**

The study evaluated the pharmacological activities of ethanolic extracts of *Lilium candidum* and *Chamomilla capitula* using validated animal models. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenolic compounds, and triterpenoids, which are known contributors to gastroprotective, neuropharmacological, and antioxidant effects. Acute toxicity studies established safety up to 2000 mg/kg, suggesting a wide therapeutic margin. Anti-ulcer activity: Both extracts significantly reduced gastric acidity and ulcer index in pyloric ligation and aspirin-induced models. The effect was dose-dependent, with *Chamomilla capitula* (400 mg/kg) showing maximum protection (~77–79%), comparable to ranitidine. Anti-diarrhoeal activity: In castor oil-induced diarrhoea and enteropooling models, both extracts decreased stool frequency and intestinal fluid accumulation. *Chamomilla capitula* at 400 mg/kg produced the strongest inhibition (~75–82%), nearly equal to loperamide. Antianxiety activity: Elevated plus maze testing showed dose-dependent increases in open-arm entries and time spent in open arms, indicating anxiolytic effects. Antidepressant activity: In forced swim and tail suspension tests, both extracts reduced immobility time significantly, with *Chamomilla capitula* (400 mg/kg) showing the most pronounced antidepressant-like effect (~73%), comparable to diazepam. Overall, both extracts demonstrated gastroprotective, anti-diarrhoeal, anxiolytic, and antidepressant properties, with *Chamomilla capitula* showing consistently superior effects at higher doses.

**Keywords:** *Chamomilla capitula,* gastroprotective, anti-diarrhoeal, anxiolytic, gastroprotective, *Lilium candidum*

## Introduction

### Plant Profile

***Lilium candidum* L. (Madonna Lily)**

Taxonomical Classification

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Liliopsida
- Order: Liliales
- Family: Liliaceae
- Genus: *Lilium*
- Species: *Lilium candidum* L.

Vernacular Names

- English: Madonna Lily, White Lily
- Hindi: Kumudini
- Arabic: Susannah
- French: Lis blanc

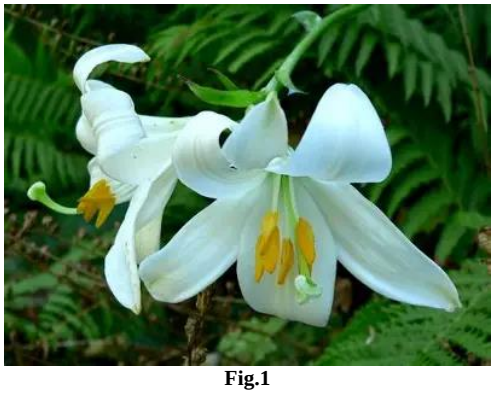

**Fig.1**

### Morphological Features

*Lilium candidum* is a perennial herb arising from an underground bulb. The stem grows up to 1–1.2 m tall, bearing whorls of lanceolate leaves. The plant produces large, fragrant, trumpet-shaped white flowers with six tepals. It thrives in temperate climates and well-drained soils.

### Distribution

Native to the Eastern Mediterranean region (Lebanon, Israel, Syria, Greece) but extensively cultivated in Europe, Asia, and North America as an ornamental and medicinal plant.

### Phytoconstituents

The plant contains alkaloids, flavonoids, glycosides, saponins, tannins, and phenolic compounds. Flavonoids and phenolic acids are believed to contribute to its antioxidant and gastroprotective properties.

### Traditional & Ethnomedicinal Uses

- Treatment of ulcers, gastritis, and inflammation.
- Wound healing and skin disorders.
- Relief of respiratory problems (cough, bronchitis).
- Sedative, diuretic, and tonic uses in folk medicine.

### Pharmacological Activities Reported

- Anti-ulcer and gastroprotective
- Antimicrobial and wound healing
- Anti-inflammatory and antioxidant
- Neuroprotective and anxiolytic

***Chamomilla capitula* (Syn. *Matricaria chamomilla* L.) (German Chamomile)**

**Taxonomical Classification**

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Asterales
- Family: Asteraceae
- Genus: *Matricaria*
- Species: *M. chamomilla* L.

**Vernacular Names**

- English: German Chamomile, Wild Chamomile
- Hindi: Babuna
- Arabic: Babunaj
- French: Camomille allemande

**Fig.2**

**Morphological Features**

An annual aromatic herb, growing up to 15–50 cm tall, with erect branched stems. The leaves are bipinnate to tripinnate with fine thread-like segments. Flower heads have yellow disc florets and white ray florets with a hollow conical receptacle. Flowers emit a sweet apple-like fragrance.

**Distribution**

Widely distributed across Europe, Western Asia, and North Africa. Cultivated worldwide for medicinal, cosmetic, and aromatic uses.

**Phytoconstituents**

The flowers contain essential oils (α-bisabolol, chamazulene), flavonoids (apigenin, luteolin, quercetin), coumarins, tannins, and phenolic acids.

**Traditional & Ethnomedicinal Uses**

- Relief of gastrointestinal disorders (flatulence, diarrhoea, ulcers).
- Used as a mild sedative and anxiolytic.
- Treatment of wounds, eczema, and skin inflammation.
- Antispasmodic and analgesic in menstrual disorders.

**Pharmacological Activities Reported**

- Gastroprotective and anti-ulcer
- Anti-diarrhoeal and antispasmodic
- Anxiolytic and antidepressant
- Antioxidant and anti-inflammatory
- Antimicrobial and wound healing

## Materials and Methods

**Plant Materials**

Fresh flowers of *Lilium candidum* and *Chamomilla capitula* were collected from local herbal gardens and authenticated by a botanist from the Department of Botany. Voucher specimens were prepared and deposited for future reference. The flowers were washed, shade-dried at room temperature, pulverized using a mechanical grinder, and stored in airtight containers until use.

**Preparation of Extracts**

The dried and finely powdered plant material was subjected to successive extraction with ethanol using a Soxhlet apparatus. The extraction process was continued until the siphoning solvent appeared colorless, indicating exhaustive extraction of soluble constituents. The resulting ethanolic extracts were concentrated under reduced pressure using a rotary evaporator to remove residual solvent. The concentrated crude extracts were then stored at 4 °C in a refrigerator until further analysis. The percentage yield of each extract was calculated relative to the initial dry weight of the plant material.

**Chemicals and Drugs**

- **Chemicals**: Ethanol, aspirin, castor oil, copper sulfate, and other analytical grade reagents (purchased from Merck, India).
- **Drugs**: Ranitidine (20 mg/kg), Diazepam (10 mg/kg), and Loperamide (5 mg/kg) were used as standard reference drugs.

**Experimental Animals**

Healthy Swiss albino mice (20–25 g) and Wistar albino rats (150–200 g) of either sex were procured from the institutional animal facility. All animals were maintained under standardized laboratory conditions, including a controlled temperature of 25 ± 2 °C, a 12-hour light/dark cycle, and unrestricted access to a standard pellet diet and potable water. The experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and conducted in strict accordance with the guidelines prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

**Preliminary Phytochemical Screening**

Qualitative phytochemical analyses were conducted on the ethanolic extracts of *Lilium candidum* and *Centaurea capitula* to identify the presence of key secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, triterpenoids, and saponins. The screening procedures followed established protocols as outlined by Harborne (1998) and Kokate (2010).

**Acute Toxicity Studies**

Acute oral toxicity of the extracts was evaluated as per OECD Guideline 423 (Acute Toxic Class Method). Female mice were administered increasing doses of extracts (300, 600, 1000, and 2000 mg/kg) orally. The animals were observed for 14 days for signs of toxicity, behavioral changes, and mortality. The $LD_{50}$ value was estimated accordingly.

**Pharmacological Studies**

**Anti-Ulcer Activity**

***a) Pyloric Ligation-Induced Ulcers***

Albino rats were fasted for 24 hours prior to the experiment, with unrestricted access to water to ensure hydration. Pyloric ligation was carried out under light ether anesthesia to induce gastric secretion. The test extracts were administered orally at doses of 200 mg/kg and 400 mg/kg, while the standard drug, ranitidine, was given at 20 mg/kg p.o., one hour before the ligation procedure. After a post-ligation period of four hours, the animals were humanely sacrificed. The stomachs were carefully excised, and gastric contents were collected for analysis.

Parameters assessed included free acidity, total acidity, and ulcer index, providing quantitative measures of the gastroprotective efficacy of the treatments.

***b) Aspirin-Induced Ulcers***

Albino rats fasted for 24 h received aspirin (200 mg/kg, p.o.) to induce ulcers. Extracts (200 and 400 mg/kg, p.o.) and ranitidine (20 mg/kg, p.o.) were administered 1 h prior to aspirin. After 4 h, animals were sacrificed, stomachs examined for ulcer index, and percentage protection calculated.

**Anti-Diarrhoeal Activity**

***a) Castor Oil-Induced Diarrhoea***

Mice fasted for 18 h received castor oil (0.5 mL, p.o.) to induce diarrhoea. Extracts (200 and 400 mg/kg, p.o.) and loperamide (5 mg/kg, p.o.) were given 1 h prior. The number of watery stools was recorded for 4 h, and percentage inhibition was calculated.

***b) Castor Oil-Induced Enteropooling***

Mice fasted for 18 h received castor oil (0.5 mL, p.o.). Extracts and loperamide were administered 1 h prior. After 4 h, animals were sacrificed, and the small intestine was isolated. The volume of intestinal content was measured, and percentage inhibition of enteropooling was calculated.

**Anti-anxiety Activity**

**Elevated Plus Maze Test**

Mice were placed individually in the center of the plus maze (two open arms, two closed arms, elevated 50 cm from the floor). Extracts (200 and 400 mg/kg, p.o.) and diazepam (10 mg/kg, i.p.) were administered 1 h prior. The number of open/closed entries and time spent in open/closed arms during a 5 min observation period were recorded.

**Antidepressant Activity**

a) Forced Swim Test (FST) Each mouse was individually placed in a transparent glass cylinder (25 cm in height, 10 cm in diameter) filled with water maintained at 25 °C to a depth of 15 cm, ensuring no contact with the bottom. The test extracts and diazepam were administered orally one hour prior to the experiment. Animals were observed for a total duration of 6 minutes, with immobility time recorded during the final 4 minutes. The reduction in immobility time was calculated and expressed as a percentage change relative to the control group.

b) Tail Suspension Test (TST) Mice were suspended by the tail using adhesive tape affixed approximately 1 cm from the tip, secured to a horizontal bar positioned 50 cm above the floor. Extracts and diazepam were administered orally one hour before testing. Each mouse was observed for a 6-minute period, and the duration of immobility was recorded. The percentage reduction in immobility time was computed to evaluate antidepressant-like activity.

**Statistical Analysis:** All data were expressed as Mean ± SEM (Standard Error of the Mean). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Dunnett's or Tukey's post hoc multiple comparison tests, as appropriate. A p-value less than 0.05 was considered indicative of statistical significance. Graph Pad Prism and/or SPSS software were employed for data analysis and graphical representation.

## Result and Discussion

**Preliminary Phytochemical Screening**

Freshly collected flowers of *Lilium candidum* and *Chamomilla capitula* were selected for phytochemical investigation. The floral material was shade-dried to preserve thermolabile constituents, finely powdered, and subjected to successive extraction using ethanol as the solvent.

**Table.1:** Physical appearance and extractive values of *Lilium candidum* and *Chamomilla capitula*

| Sl. No | Drug | Solvent | Colour | % Yield |
|---|---|---|---|---|
| 1 | *Lilium candidum* | Ethanol | Dark Brown | 30–40% |
| 2 | *Chamomilla capitula* | Ethanol | Dark Brown | 30–35% |

**Table.2:** Qualitative phytochemical analysis of ethanolic extracts

| Test | *L. candidum* | *C. capitula* |
|---|---|---|
| **Alkaloids** | | |
| Mayer's test | + | – |
| Wagner's test | + | + |
| Dragendorff's test | – | – |
| Hager's test | + | – |
| **Triterpenoids** | | |
| Salkowski's test | + | + |
| Libermann–Burchard test | – | + |
| **Saponins** | | |
| Froth test | – | – |
| Libermann–Burchard test | + | – |
| **Flavonoids** | | |
| Alkaline reagent test | + | + |
| Lead acetate test | – | – |
| **Phenolic & Tannins** | | |
| Ferric chloride test | + | + |
| Gelatin test | + | + |
| Lead acetate test | – | + |
| Alkaline reagent test | + | – |
| Vanillin hydrochloride | + | – |

(+ = present; – = absent)

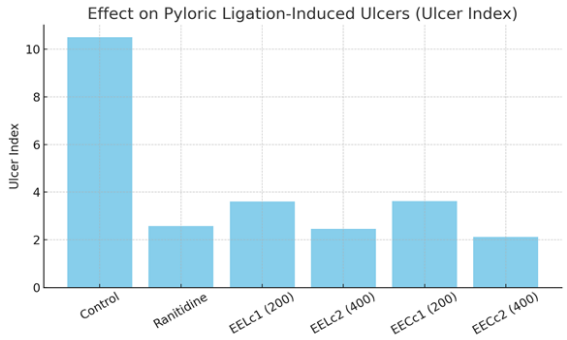


**Fig.3:** Pyloric ligation-induced ulcers (Ulcer index)

**Acute Toxicity Assessment**

Acute oral toxicity studies demonstrated that the ethanolic extracts of *Lilium candidum* and *Chamomilla capitula* were well tolerated up to a dose of 2000 mg/kg body weight. No mortality or observable signs of toxicity were recorded throughout the study duration. The estimated median lethal dose ($LD_{50}$) was determined to be greater than 2000 mg/kg, indicating a favorable safety profile for subsequent pharmacological evaluations.

**Evaluation of Anti-Ulcer Activity**

***Effect on Pyloric Ligation-Induced Ulcers***

Ethanolic extracts of *Lilium candidum* (EELc) and *Chamomilla capitula* (EECc), at doses of 200 and 400 mg/kg, significantly reduced ulcer index, free acidity, and total acidity compared to the control group. The effects were dose-dependent, and at 400 mg/kg, both extracts produced protection comparable to standard ranitidine (20 mg/kg).

**Effect on Aspirin-Induced Ulcers**

In aspirin-induced ulcer model, both extracts showed significant protective activity. Maximum protection was observed with EECc2 (400 mg/kg, 77.41%) and EELc2 (400 mg/kg, 72.05%), while ranitidine exhibited 81.53% ulcer protection.

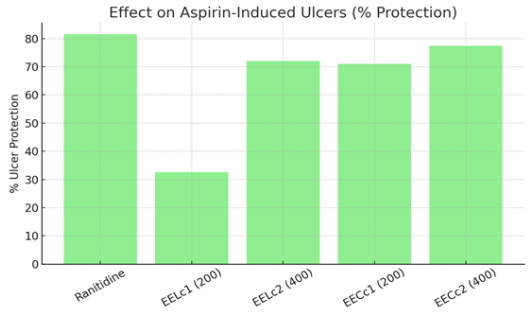


**Fig.4:** Aspirin-induced ulcers (% ulcer protection)

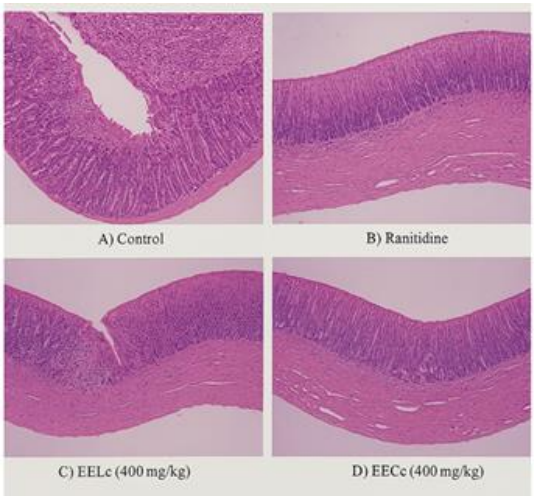


**Fig.5:** Histopathological Assessment

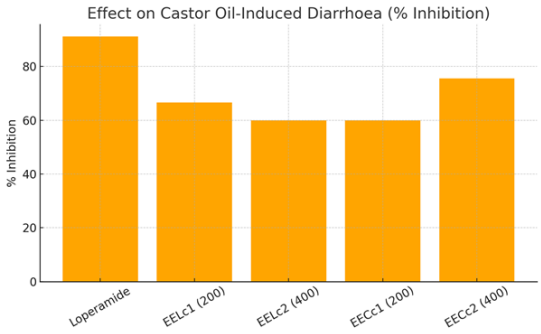


**Fig.6:** Castor oil-induced diarrhoea (% inhibition)

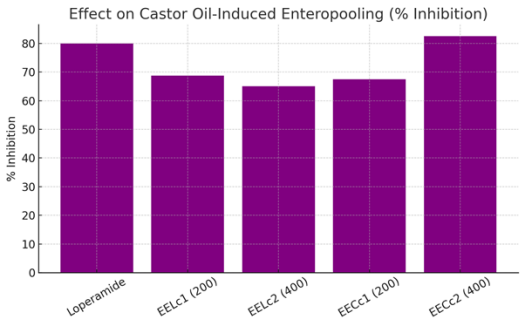


**Fig.7:** Castor oil-induced enteropooling (% inhibition)

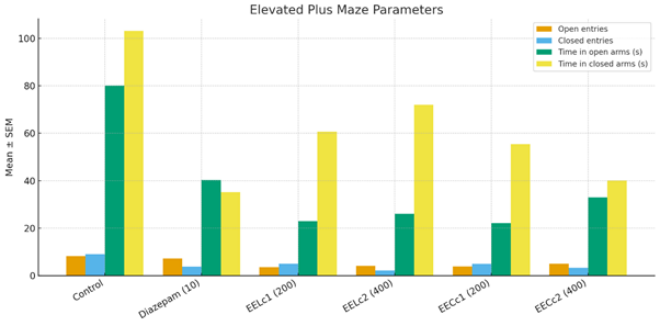


**Fig.8:** The Elevated Plus Maze parameters

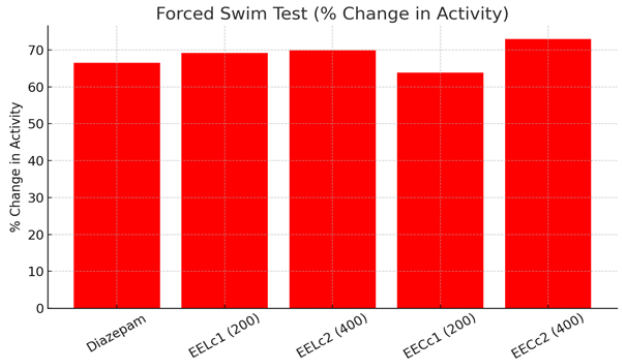


**Fig.9:** FST (% change in activity)

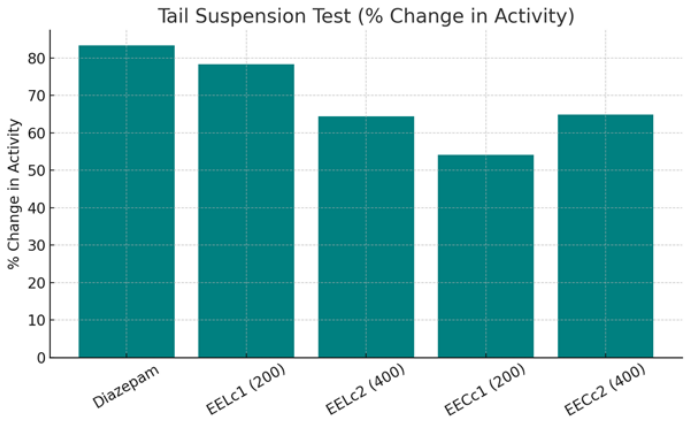


**Fig.10:** Tail suspension test (% change in activity)

## Discussion

### Preliminary Phytochemical Screening

The preliminary phytochemical analysis of *Lilium candidum* and *Chamomilla capitula* extracts confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds. These secondary metabolites are well-known for their diverse pharmacological effects. Alkaloids and flavonoids are often implicated in gastroprotective and neuropharmacological actions, while tannins and phenolic compounds contribute to antioxidant and anti-ulcer activities. The detection of triterpenoids also supports potential anti-inflammatory and anti-stress properties. The findings from phytochemical screening thus provide a biochemical rationale for the observed pharmacological effects in subsequent models.

### Acute Toxicity Studies

The safety evaluation of the ethanolic extracts revealed no signs of toxicity or mortality up to 2000 mg/kg, suggesting a wide margin of safety and supporting their potential therapeutic use.

This is consistent with traditional usage of these plants in folk medicine and validates their biocompatibility at pharmacologically relevant doses.

**Anti-Ulcer Activity**

Both pyloric ligation and aspirin-induced ulcer models demonstrated significant gastroprotective activity of the extracts. *Lilium candidum* and *Chamomilla capitula* extracts, especially at 400 mg/kg, significantly reduced free acidity, total acidity, and ulcer index compared to control, with protection approaching that of standard ranitidine. The higher dose of *Chamomilla capitula* (EECc2) offered maximum protection (79.8% in pyloric ligation and 77.4% in aspirin-induced model), indicating strong anti-ulcer potential. The observed effects may be attributed to the presence of flavonoids and tannins, which are known to strengthen gastric mucosal defense, inhibit acid secretion, and enhance cytoprotective mechanisms.

**Anti-Diarrhoeal Activity**

In castor oil-induced diarrhoea and enteropooling models, both extracts showed significant inhibition of diarrhoeal episodes and intestinal fluid accumulation. EECc2 demonstrated the highest effect (75.55% inhibition of diarrhoea and 82.5% inhibition of enteropooling), comparable to loperamide. These findings suggest that the extracts reduce intestinal hypersecretion and motility, likely mediated through tannins and flavonoids that possess anti-secretory and spasmolytic actions. This aligns with ethnomedicinal claims of these plants in treating gastrointestinal disturbances.

**Antianxiety Activity**

The elevated plus maze results indicated that both extracts, particularly at higher doses, produced anxiolytic-like effects, evident from increased open-arm entries and longer time spent in open arms. Although the effects did not surpass diazepam, EELc2 and EECc2 showed marked improvements compared to lower doses. The anxiolytic potential could be linked to alkaloids and flavonoids, which interact with GABAergic and serotonergic systems, thereby modulating anxiety-related behaviors.

**Antidepressant Activity**

In both forced swim and tail suspension tests, the extracts significantly reduced immobility time, demonstrating antidepressant-like effects. EECc2 produced the maximum reduction in immobility (72.95%), which is comparable to standard diazepam. The antidepressant activity may be attributed to flavonoids and phenolic compounds, which have been reported to enhance monoaminergic transmission and exert neuroprotective effects. The dose-dependent efficacy observed further confirms the pharmacological relevance of the phytochemicals detected.

**Overall Interpretation**

The present study demonstrates that ethanolic extracts of *Lilium candidum* and *Chamomilla capitula* possess significant gastroprotective, anti-diarrhoeal, anxiolytic, and antidepressant activities. Among the two, *Chamomilla capitula* at higher dose (400 mg/kg) consistently showed stronger pharmacological effects, which may be due to a higher concentration or synergistic activity of active phytoconstituents. The results validate the ethnomedicinal uses of these plants and highlight their potential as sources of bioactive compounds for drug development targeting gastrointestinal and neuropsychiatric disorders.

## Conclusion

The findings provide scientific validation of traditional medicinal claims regarding *Lilium candidum* and *Chamomilla capitula*. Both extracts exhibited strong safety, gastrointestinal protection, and neuropharmacological benefits, highlighting their potential as natural therapeutic agents.

- *Chamomilla capitula*, particularly at higher doses, demonstrated the most promising pharmacological profile, suggesting it may be a valuable candidate for developing treatments for ulcerative, diarrhoeal, and neuropsychiatric disorders.
- The presence of multiple phytoconstituents likely contributes to their synergistic therapeutic effects.
- Further studies on isolation, structural characterization, and mechanistic pathways of active compounds are warranted to advance these findings toward clinical applications.

## Conflict of Interests

The authors declare no conflict of interest

**Ethics Approval**: Not applicable

**Funding:** This study received no specific funding from public, commercial, or not for profit funding agencies.

## AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

## Data Availability

Data will be available on request

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